REMARKS/ARGUMENTS

Claims 1 and 19 are amended and claims 27-29 are newly added.

I.

Support for Amendments

Amendments to the claims are supported throughout the application including the specification and claims as originally filed and contain no new matter. Specific support is indicated for each amendment for the convenience of the examiner.

Claims 1 is amended to newly recite a method of amplifying gene expression in a moss plant cell by increasing the copy number of integrated transforming DNA constructs and regenerating the transformed moss plant cell into moss protonema comprising a plurality of copies of said at least one heterologous nucleotide sequence. Support may be found at pages 3 and 4, which provides,

> "The skilled addressee will appreciate that once the said at least two heterologous constructs are transformed into the moss plant cell, such as a moss protoplast, for example a Physcomitrella patens protoplast, which is then permitted to regenerate into moss protonema, for example Physcomitrella patens, they will undergo recombination with each other many times over. This process, once initiated in the moss plant cell, increases the copy number of integrated transforming DNA constructs of the invention therein."

Claims 1 and 19 are also amended to recite, "providing at least a second heterologous nucleic acid construct different from the first." Support may be found for instance, at pages 18 and 19, which shows a first construct having a 5' first recombination sequence including a 250 bp sequence a 3' second recombination sequence including a 208 bp sequence. A second construct is provided on pages 19-20, which shows a 5' second recombination sequence including a 208 bp sequence and a 3' first recombination sequence including a 250 bp sequence.

Claims 1 and 19 are also amended to clarify that the first recombination sequence differs from the second recombination sequence and the first and second recombination sequences form a set of recombination sequences designed to enable said at least first and said at least second constructs to recombine with each other *in vivo*. Support may be found as provided above. Further support may be found at page 3, line 32 through page 4, line 3, which provides,

> "The skilled addressee will appreciate that once the said at least two heterologous constructs are transformed into the moss plant cell... they will undergo recombination with each other many times over."

Still further support is found at page 4, lines 19-22, which provides in part, "The at least first and the at least second recombination sequences form a complementary set that make it possible for the constructs of the invention to recombine with each other."

Claims 27 and 28 are newly added, depend from claims 1 and 19 respectively, and add that the set of recombination sequences enable integration of heterologous sequences obtained from recombined at least first and at least second constructs into the moss plant cell's genome. Support may be found at page 15, lines 1-6, which provides,

> "A discussed above, the present inventors show that enhanced expression from constructs of the invention introduced (preferably at high levels) into the protoplasts of moss, preferably at high cell density, such as *Physcomitrella patens*, which constructs are integrated into the genome give rise to transcribed mRNA."

Further support may be found at page 13, lines 21-26, which provide,

> "Thus, by employing the at least two constructs of the invention as hereindescribed production lines may be generated harbouring high copy numbers of the target gene which in turn results in high protein yields over the cultivation period in a suitable bioreactor."

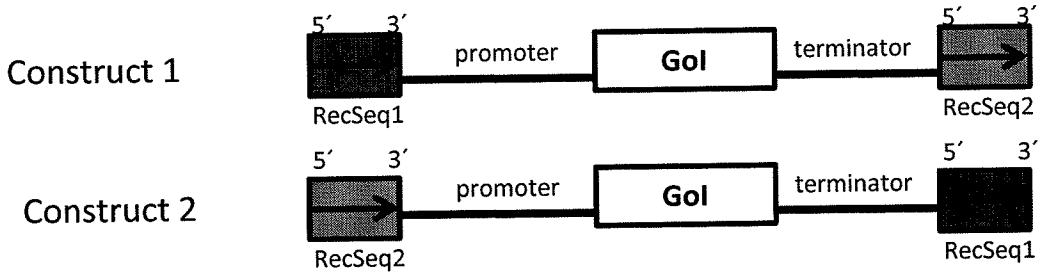
Claim 29 is newly added, depends from claim 19 and adds that recombination sequences are selected from the group consisting of genomic DNA, cDNA, an intron, a non-coding region or an exon, and a combination thereof. Support may be found in claim 4.

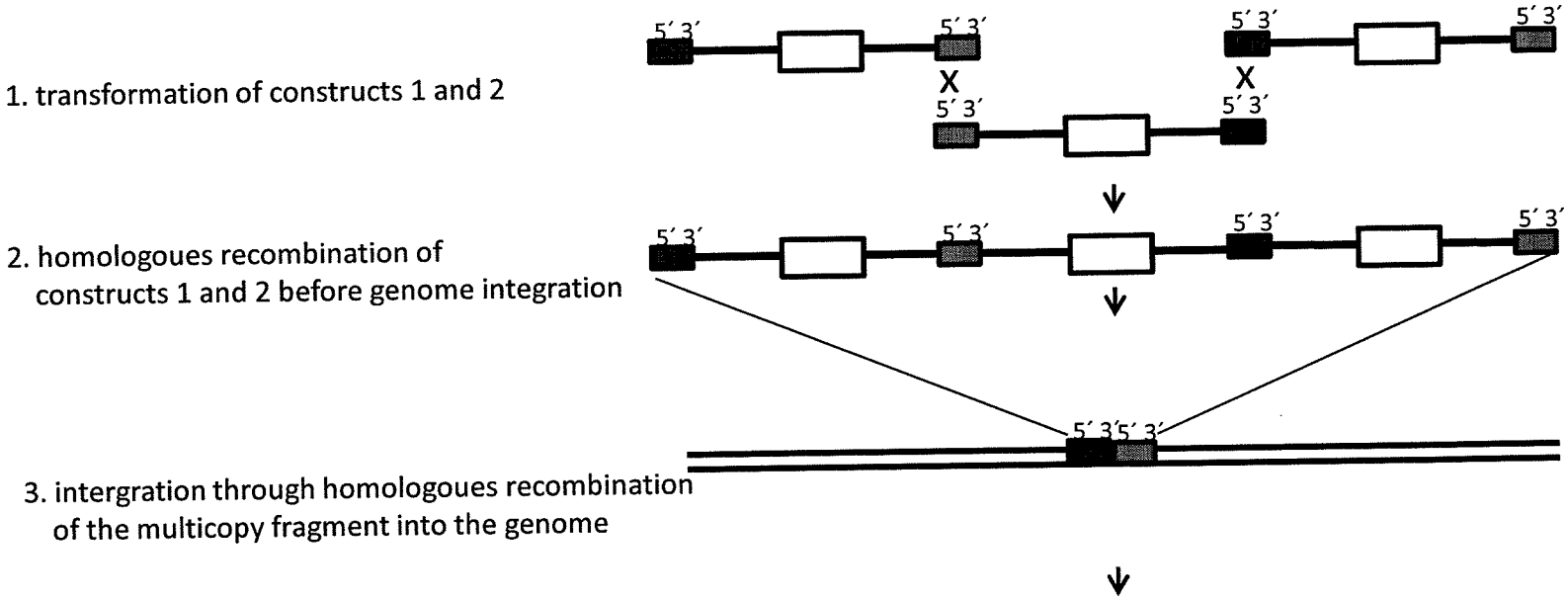
II.

Introduction to the Invention

The present invention provides methods and systems for amplifying gene expression in a transformed moss plant cell. Broadly, the invention includes at least two constructs, each having at least one heterologous nucleotide sequence. The first construct is flanked at the 5' end by a first recombination sequence and is flanked at the 3' end by a second recombination sequence. The second construct is flanked at the 5' end by the second recombination sequence and is flanked at the 3' end by the first recombination sequence. This configuration enables the constructs to recombine with each other. The inventors surprisingly found such a system results in an increase in the integrated copy number of heterologous nucleic acid constructs in regenerated tissue which in turn correlated with increased protein expression levels.

A schematic overview of this general technical approach is summarized below. Referring to the below diagram showing a preferred embodiment, two constructs are created, each construct including a gene of interest operably linked to a promoter and a terminator sequence; each construct flanked by recombination sequences RecSeq1 and RecSeq2. More specifically, two variants of the operational gene are constructed. The first is flanked at the 5' end with recombination sequence 1 (RecSeq1) and flanked at the 3' end with recombination sequence 2 (RecSeq2). The second is flanked at the 5' end with recombination sequence 2 (RecSeq2) and flanked at the 3' end with recombination sequence 1 (ReqSeq1). The moss cell is transformed with both constructs and homologous recombination occurs between the constructs to form a multicopy product. After this first recombination step, the multicopy product is integrated into the moss cell genome, in this case preferably via homologous recombination.


Construct 1
5′ 3′
promoter
GoI
terminator
5′ 3′
RecSeq1
RecSeq2
Construct 2
5′ 3′
promoter
GoI
terminator
5′ 3′
RecSeq2
RecSeq1


1. transformation of constructs 1 and 2
2. homologoues recombination of constructs 1 and 2 before genome integration
3. intergration through homologoues recombination of the multicopy fragment into the genome
5′ 3′
X

III.

Response to Rejections Under 35 USC 112, 2nd paragraph

Claims 1-8 and 19-26 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

The definiteness of language employed must be analyzed not in a vacuum, but in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing ordinary skill in the art. Allen Archery Inc. v. Browning Mfg. Co., 2 USPQ2d 1490, 1494 (Fed. Cir. 1987). "If the claims, read in light of the specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." North Am. Vaccine, Inc. v. American Cyanimid Co., 28 USPQ2d 1333, 1339 (Fed. Cir. 1993).

A. Claims 1 and 19 are amended from "a complementary set of recombination sequences" to "a set of recombination sequences" for clarity

With respect to claims 1 and 19, and thus claims 2-8 and 20-26 by dependence, the examiner objects to the term "complementary" in relation to the formation of a set of complementary recombination sequences from the first and second recombination sequences. Specifically, the examiner considers the recitation confusing since the term is often used in molecular biology to refer to the binding of base pairs between nucleic acid strands.

To avoid confusion, the term "complementary" is deleted and thus the claims recite that the first and second recombination sequences form a set of recombination sequences. Accordingly, Applicant respectfully requests the rejections be withdrawn and the claims allowed.

B. Claims 1 and 19 are amended to delete the " same orientation" language

With respect to claims 1 and 19, and thus claims 2-8 and 20-26 by dependence, the examiner objects to the phrases " a second recombination sequence in the same orientation as the

first" and "said first recombination sequence in the same orientation as the second."

To avoid confusion, the "same orientation" language is deleted. Accordingly, Applicant respectfully requests the rejections be withdrawn and the claims allowed.

IV.

Response to Claim Rejections Under 35 U.S.C. §102 (Anticipation)

Anticipation requires a single prior art reference disclose each and every element of the claim under consideration." W.L. Gore & Assocs. V. Garlock, Inc., 220 USPQ 303, 313 (Fed. Cir.1983). However, it is not enough that the reference discloses all of the claimed elements in isolation. Rather, as stated by the Federal Circuit, the prior art reference must disclose each and every element of the claimed invention "arranged as in the claim." Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co., 730 F.2d 1452, 221 USPQ 481, 485 (Fed. Cir.1984). Further, the prior art must be such that a person of ordinary skill in the art would consider there to be no difference between the claimed invention and the referenced disclosure. In re Gurley, 27 F.3d 551, 31 USPQ2d 1130, 1132 (Fed Cir. 1994). For brevity the references are compared to the claims as amended.

A. Claims 19 and 20 are not anticipated by Hartley et al. (US 2006/0035269)

Claims 19 and 20 stand rejected under 35 U.S.C. § 102(e) as being anticipated by Hartley et al. (incorrectly referred to as Perkins in the Office Action). The examiner cites Hartley et al. as teaching a vector (pEZC726) including coding regions of the cam and kan genes. The genes are separated by first and second recombination sequences, namely attP and loxP, such that each is positioned at either a 5' end or 3' end of the coding regions. Thus, the examiner concludes that two molecules of pEZC726 would provide the features set forth in the claims and thus anticipate the invention.

A1. Claim 19 as amended provides two different nucleic acid constructs; whereas providing two copies of a same plasmid disclosed in FIG. 2C of Hartley et al. would not provide two different constructs

Claim 19, from which claim 20 depends, is amended to clarify that the first and second heterologous nucleic acid constructs are different. That is, the constructs themselves are identifiable from one another. For instance, in the present invention a first construct includes a heterologous nucleotide sequence operably linked to a promoter and flanked at the 5' end thereof by a first recombination sequence and flanked at the 3' end by a second recombination sequence. A second heterologous nucleic acid construct includes a heterologous nucleotide sequence operably linked to a promoter and flanked at the 5' end thereof by the second recombination sequence and flanked at the 3' end by the first recombination sequence.

On page 4 of the Office Action, the examiner proposes providing two copies of the plasmid provided in FIG. 2C since there are two heterologous sequences (cam and kan) separated by attP and loxP. That is, attP may act as a 5' sequence for cam and as a 3' sequence for kan; and loxP may act as a 3' sequence for cam and as a 5' sequence for kan. Accordingly, the examiner proposes two molecules of pEZC726 may constitute a "set" of vectors as claimed. While there may be different inducible regions within the single plasmid, only a single plasmid is provided. For instance, if possessing two copies of the same pEZC726, the two are not discernable. As such, Hartley et al. do not provide two different constructs as set forth in the amended claims. Accordingly, Applicant respectfully requests the rejection be withdrawn and the claims allowed.

A2. The vectors recited in claims 19 and 20 offer a different technical approach than that provided in Hartley et al., namely claims 19 and 20 utilize two different recombination sequences between two constructs whereas Hartley et al. require four different recombinational sites between two DNA vectors

As introduced above, the present invention when operating with two constructs uses two different recombination sequences. In summary, the first recombination sequence is provided as

the 5' flanking sequence in the first construct and as the 3' flanking sequence in the second construct; and the second recombination sequence is provided as the 3' flanking sequence in the first construct and as the 5' flanking sequence in the second construct. This provides a different technical approach than that taken by Hartley et al.

The object of Hartley et al. is to improve recombinational cloning using engineered recombination sites. Recombinational cloning is provided by the use of nucleic acids, vectors and methods for moving or exchanging segments of DNA molecules using engineered recombination sites and recombination proteins to provide chimeric DNA molecules that have the desired characteristic(s) and/or DNA segment(s). In particular, Hartley et al. relates to the provision of an alternative subcloning system that provides advantages over the known use of restriction enzymes and ligases. This technology makes use of site specific recombinases which are enzymes that are present in some viruses and bacteria and have been characterized to have both endonuclease and ligase properties. These recombinases recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (para [0005]). Numerous recombination systems from various organisms have been described (para . [0006]) and many of these belong to the integrase family of recombinases, the best studied of which are the (i) Integrase/att system from bacteriophage λ, the (ii) Cre/loxP system from bacteriophage P1, and the (iii) FLP/FRT system from the *Saccharomyces cerevisiae* 2μ circle plasmid (para. [0007]).

Procedurally, beginning at para. [0037], the method of making chimeric DNA includes combining (i) an Insert Donor molecule (e.g. pEZC705), comprising a desired DNA segment flanked by a **first** recombination site and a **second** recombination site, wherein the first and second recombination sites do not recombine with each other; (ii) a Vector Donor DNA molecule containing a **third** recombination site and a **fourth** recombination site, wherein the third and fourth recombination sites do not recombine with each other; and (iii) one or more site specific recombination proteins capable of recombining the **first and third** recombinational sites and/or the **second and fourth** recombinational sites. The requirement of using four different sites is emphasized at para. [0121] of Hartley et al. explaining Fig. 1 when stating: "The square and circle are different sets of recombination sites (e.g. lox sites or att sites)". It is thus clear that

mutant forms of lox and att are used which is explained in detail in the specification.

Since a system requiring four different recombinational sites comprised by two different DNA vectors does not teach a system requiring only two different recombination sequences comprised by two different constructs, Hartley et al. do not anticipate the invention as arranged in the claims. Accordingly, Applicant respectfully requests the rejection be withdrawn and the claims allowed.

A3. The set of nucleic acid vectors recited in claims 19 and 20 include two constructs that recombine with each other *in vivo* for amplifying gene expression in a moss plant cell, whereas the plasmids of Hartley et al. would not recombine with each other *in vivo* for amplifying gene expression in a moss plant cell

Clams 19 and 20 provide that the recombination sequences are designed to enable the at least first and at least second constructs to recombine with each other *in vivo*. This *in vivo* recombination amplifies gene expression in the moss plant cell as set forth in the preamble of claim 19.

Differences between the constructs themselves and the technical approaches taken by Hartley et al. compared to claims 19 and 20 are discussed above. For completeness, the set of nucleic acid vectors as proposed by the examiner would not recombine with each other *in vivo* for amplifying gene expression in a moss plant cell.

Referring to the discussion above, Hartley et al. make use of site specific recombinases which are enzymes that are present in some viruses and bacteria and have been characterized to have both endonuclease and ligase properties. Again, these recombinases recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. Due to the origin of the different recombinant proteins and recombinational sites belonging to the above integrase family of recombinases, it should readily be evident to a skilled artisan that these sequences would not enable integration into the moss plant cell for expression.

Accordingly, Hartley et al. do not anticipate the present invention and Applicant respectfully requests the rejection be withdrawn and the claims allowed.

B. Claims 1-8, 19-22, 24 and 25 are not anticipated by Zeidler et al.

Claims 1-8, 19-22, 24 and 25 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Zeidler et al. (J. Plant Physiol. 154:641-650 (1999)).

The examiner refers to FIG. 6C to demonstrate the presence of at least two heterologous sequences linked to promoters in the same orientation, flanked by a 5' and 3' recombination sequence. The examiner concludes two such plasmids are present in some transformants and thus in some cases, the claim limitations are met.

Zeidler et al. do provide at least two heterologous sequences linked to promoters; however, Zeidler et al:

a) do not provide two different constructs as set forth by the present invention,
b) do not provide an *in vivo* system where a first recombination sequence is positioned at the 5' end of a first hetereologous nucleotide sequence and the 3' end of a second heterologous nucleotide sequence and a second recombination sequence positioned at the 3' end of the first heterologous nucleotide sequence and at the 5' end of the second heterologous sequence,
c) do not teach such recombination sequences enable the constructs to recombine *in vivo* with each other as set forth in the claims, and
d) do not teach increasing the copy number of integrated transforming DNA constructs and regenerating the moss plant cell into moss protonema.

Each distinction is briefly discussed in comparison to the claims as amended.

B1. Claims 1 and 19 as amended provide two different nucleic acid constructs; whereas providing two copies of a same plasmid disclosed in FIG. 6C of Zeidler et al. would not provide two different constructs

Claims 1 and 19 are amended to clarify that the first and second heterologous nucleic acid constructs are different. That is, the constructs themselves are identifiable from one another. For instance, in the present invention a first construct includes a heterologous

nucleotide sequence operably linked to a promoter and flanked at the 5' end thereof by a first recombination sequence and flanked at the 3' end by a second recombination sequence. A second heterologous nucleic acid construct includes a heterologous nucleotide sequence operably linked to a promoter and flanked at the 5' end thereof by the second recombination sequence and flanked at the 3' end by the first recombination sequence.

On page 5 of the Office Action, the examiner proposes providing two copies of the plasmid provided in FIG. 6C since there are two heterologous sequences linked to promoters in the same orientation, flanked by a 5' and 3' recombination sequence; however, while there may be different inducible regions within the plasmid, only a single construct is provided. For instance, if possessing two copies of the same pBluescript construct, the two are not discernable. As such, Zeidler et al. do not provide two different constructs as set forth in the claims. Accordingly, Applicant respectfully requests the rejection be withdrawn and the claims allowed.

B2. Claims 1 and 19 provide that the at least first construct includes a first recombination sequence at a 5' end and a second recombination sequence at a 3' end and the at least second construct includes the second recombination sequence at a 5' end and the first recombination sequence at a 3' end; however, in the rejection the examiner fails to identify the first and second recombination sequences in Zeidler et al.

As discussed above, recombination events between the two constructs in the present invention are performed in part due to the structural configuration of the recombination sequences. That is, a first recombination sequence is provided at a 5' end of a first construct and a 3'end of a second construct, and the second recombination sequence is provided at a 3' end of the first construct and a 5' end of the second construct.

In the rejection the examiner points to FIG. 6C, which provides a schematic of a single pBluescript showing GUS and HYG probes but does not indicate where the recombination sequences are found or what recombination event occurs. As such, it is unclear how the single plasmid in FIG. 6C anticipates the present invention. Accordingly, Applicant respectfully requests the rejections be withdrawn and the claims allowed.

B3. Claims 1 and 19 provide a set of recombination sequences designed to enable the at least first and at least second constructs to recombine with each other *in vivo*; however, the examiner has not demonstrated two constructs that undergo homologous recombination in Zeidler et al.

Again, an anticipation rejection requires the prior art reference teach each element "arranged as in the claims." Lindemann Machinenfabrick at 485. Structurally, the configuration of Applicant's recombination sequences is discussed above. Functionally, the result is the generation of a multicopy arrangement of the heterologous sequence of interest. This multicopy construct is subsequently incorporated into the host cell genome. An overview of the method is depicted in the introduction.

In contrast, Zeidler et al. do not generate a multicopy arrangement of a heterelogous sequence through recombination. Instead, Zeidler et al. provide a different technical approach to increase expression of heterologous sequences. The object of Zeidler et al is to describe an optimized transformation procedure for the species *Ceratodon purpureus*. The use of various selectable (HPT, NPT) and screenable (GUS, LUC, GFP) reporters was established and different expression vectors were constructed for both constitutive (P-*Actin I*) and tetracycline-regulated (P-*Top 10*) gene expression. Since use of the CaMV 35S promoter only gave little or no expression, the authors screened numerous alternative promoters in transient assay, then optimized the transformation system using the strongest one. Selectable markers and a convenient vector for constitutive expression of transgenes were then developed. Building upon the recombinant tetracycline repressor :: VP 16 activator system for *Physcomitrella* a vector appropriate for switchable expression in *Ceratodon* is also described.

"Switchable" expression systems allow regulation of gene expression e.g. at a particular developmental stage, in a specific tissue or organ, and for a specified duration. These regulatable transgene systems provide for easily controlled, conditional induction or repression of expression and are indispensable tools in biochemical and agricultural research and biotechnology. Several such systems have been developed for eukaryotes. Most of these rely on the administration of either exogenous chemicals or heat shock.

The different constructs created for both transient and 'stable' expression studies used

different promoters and reporters, wherein "stable" refers to clones which are still resistant after prolonged periods of non-selective culture but does not indicate stable integration into the host cell genome. After pActin-1 has been proven to allow suitable expression, a pActin 1-GUS construct was used with transient MUG assays to optimize the transformation procedure for *Ceratodon*. As a result, PEG and magnesium concentrations in combination with an optimized heat shock treatment turned out to be the decisive interacting factors that affect transformation efficiency.

For experiments using *trans*-acting plasmids (e.g. the activator and operator plasmids of the tetracycline-regulated expression system) the equal uptake of two different DNA molecules is required. While switchable promoters are useful for the over-expression of genes regulating development, they also allow true control experiments, as genetically identical cells can be investigated with and without transgene expression, thus eliminating the problems arising from positional effects and from empty-vector "controls". Accordingly, the transformation protocol required the introduction of two different plasmids, one carrying the operator (e.g. GUS, LUC) and the other providing the activator of the modified Tc-regulated expression system.

Although the article might be regarded beneficial for the further use of *Ceratodon purpureus* in functional transgene expression studies, it does not disclose the use of two constructs with the technical features as claimed. Further, while the gene products encoded by these two constructs specifically interact on a protein level, nothing is disclosed with regard to the specific design of two constructs carrying a set of sequences that enable these constructs to homologously recombine with each other *in vivo* in order to build a multicopy nucleotide sequence that is integrated into the host cell genome.

Thus, Zeidler et al. not only lack the elements as set forth above but also represent a different technical approach to protein expression. Accordingly, it can not be said that Zeidler et al. provide the elements of the present invention "arranged as in the claims." Accordingly, Applicant respectfully requests the rejections be withdrawn and the claims allowed.

B4. Amended claim 1 includes increasing the copy number of integrated transforming DNA constructs and regenerating the moss plant cell into moss protonema comprising a plurality of copies of said at least one heterologous nucleotide sequences; whereas, Zeidler et al. do not

The present invention comprises increasing the copy number of integrated transforming DNA constructs and regenerating the moss plant cell into moss protonema. The resulting protonema includes a plurality of copies of said at least one heterologous nucleotide sequence, which increases expression. Referring to the above discussions, the copy number of integrated transforming DNA constructs is increased via recombination between the at least two constructs, followed by insertion of the multicopy product into the moss cell's genome. An exemplary schematic of this system is provided in the introduction. After which, the moss plant cell is regenerated into the thread-like chain of cells characteristic of protonema. As such, the protonema are developed having multiple copies of said at least one heterologous nucleotide sequence for increased expression.

In contrast, Zeidler et al. provide an *in vitro* or cell based transgene system that lacks integration of constructs into the host cell's genome. Further, since Zeidler et al. provides an *in vitro* or cell based system, Zeidler et al. do not regenerate transformed moss plant cells into moss protonema having multiple copies of said at least one heterologous nucleotide sequence for increased expression.

Accordingly, Applicant respectfully requests the rejection be withdrawn and the claims allowed.

V.

Response to Claim Rejections Under 35 U.S.C. §103 (Obviousness)

Claims 23 and 26 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Zeidler et al. as set forth above in view of Schaefer (Ann. Rev. Plant Biol. 2002, 53:477-501).

The examiner incorporates the above description of Zeidler et al. but acknowledges that Zeidler et al. do not teach the transformed moss is *Physcomitrella patens*. However, the

examiner relies on Schaefer as teaching transformation of the moss *Physcomitrella patens* with a heterologous nucleic acid plasmid. Accordingly, the examiner considers the substitution of *Ceratodon purpureus* with *Physcomitrella patens* to be an obvious variation as there would be a reasonable expectation of success of performing the method of Zeidler et al. in *Physcomitrella patens*.

A proper obviousness rejection requires consideration of the factual inquiries provided in Graham v. John Deere Co., 38 U.S. 1, 148 USPQ 459 (1966), including: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the prior art and the claims at issue; 3) resolving the level of ordinary skill in the pertinent art; and 4) considering the objective evidence of nonobviousness. Although Graham v. John Deere requires that certain factual inquiries be conducted to support a determination of the issue of obviousness, the actual determination of the issue requires an elevation in light of the findings in those inquiries as to the obviousness *of the claimed invention as a whole*, not merely the differences between the claimed invention and the prior art. Lear Siegelr, Inc. v. Aeroquip Corp., 221 USPQ 1025, 1033 (Fed. Cir. 1984). Further, the teachings of a prior art reference are underlying factual questions in the obviousness inquiry. Para-Ordnance Mfg., Inc. v. SGS Imp. Int'l, Inc. 73 F.3d 1085, 1088 (Fed. Cir. 1995).

Schaefer does teach the transformation of the moss *Physcomitrella patens* with a heterologous nucleic acid molecule; however, Schaefer does not provide the deficiencies of the rejection of claims 1 or 19 as set forth above. Specifically, Schaefer does not provide a system for homologous recombination between constructs. Further, Schaefer does not provide a first heterologous nucleic acid construct having at least one hetereologous nucleotide sequence, operably linked to a promoter and flanked at the 5' end by a first recombination sequence and at the 3' end by a second recombination sequence; and a second construct different from the first, operably linked to a promoter and having a second heterologous sequence flanked at the 5'end by the second recombination sequence and at the 3' end by the first recombination sequence.

Instead, Schaefer discloses homologous recombination between a construct and the genome. As such, claims 23 and 26 are not obvious over Zeidler in view of Schaefer.

Accordingly, Applicant respectfully requests the rejection be withdrawn and the claims allowed.

VI.

Conclusion

In view of the amendments and arguments set forth above, applicants respectfully request all rejections be withdrawn and all claims allowed.

Respectfully submitted,

4/15/2010

Date

Raymond Wagenknecht
Reg No. 50948

Biotech Beach Law Group PC
5677 Oberlin Dr. Ste 204
San Diego, CA 92121
(858) 587-2510